

From: OSEI CORP  
To: Ragan Broyles/R6/USEPA/US@EPA; James Staves/R6/USEPA/US@EPA; Steve Mason/R6/USEPA/US@EPA  
Subject: FW: OSEI 3 main types of bioremediation mode of action and definitions  
Date: 08/09/2012 04:28 PM  
Attachments: TYPES OF BIOREMEDIATION SECTION\_RRT VI Guidance Insert.pdf  
Category EA indexed toxicity tests for RRT VI Science committee sub committee 8 10 12docx.pdf  
Category EA indexed toxicity tests for RRT VI Science committee sub committee 8 10 12docx.pdf

Dear Ragan Broyles, Steve Mason and Jim Staves,

You can see below the incomplete information on category EA that has now been filled in and defined. The request for immediate concurrence for the use of OSE II in the Gulf for the Deep water horizon, Macondo 252 block spill has not been returned to us. You made the statement you could not find a reason not to use OSE II, therefore we want our request to concur for the use of OSE II fulfilled by the EPA/RRT VI immediately. There is oil, tar mats and tar balls coming ashore everyday in large quantities, and OSE II is the means to remediate these oil spill problems and remove them from the environment in accordance with the Clean Water Act, OPAH 90 and its revisions of 1994.

The OSEI Corporation has backed up its use with efficacy testing, toxicity testing on salt and fresh water marine species, toxicity to responders, and have shown through testing there is nothing harmful in OSE II. A law suit this week has now been filed on the US EPA in regards to the use of dispersants, you have a non toxic NCP listed product that numerous stake holders and the responsible party wanted used and the EPA has been the stumbling block, we are now requesting you to remove yourselves as the stumbling block and concur with the use of OSE II immediately.  
Steven Pedigo

From: stevenosei@msn.com  
To: michael.baccigalopi@glo.texas.gov  
Subject: OSEI 3 main types of bioremediation mode of action and definitions  
Date: Thu, 9 Aug 2012 13:32:41 -0500

Dear Michael,

Enclosed please find the definitions and mode of actions of the three types of Bioremediation for inclusion in the overall Bioremediation Guidance document you referenced and sent earlier as: 'Biorem Draft 1'.

I recommend that the section entitled Types of Bioremediation be moved up to follow the Purpose, placing it before Characteristics of Spilled Oil and Characteristics of Affected Habitats as currently listed in your Draft Doc table of contents.

Included also is an added appendix for the guidance document that lists references to toxicity tests done on EA Type Bioremediation.

Please advise as to next steps with the sub-committee, timeline and/or meeting schedule as I am not clear on the framework of this activity and want to ensure I can schedule full participation. I am well in progress reviewing the full Bioremediation draft document, and if you have any later versions please forward. I estimate I will be complete on my review and suggested additions for the entire doc by end of August. I would appreciate it if you would add the appendix number to work with the guidance document, so there is no crossover. The documents are attached and included below in case the attachments will not go through as in the past.

## TYPES OF BIOREMEDIATION, CATEGORY DEFINITIONS

### AND MODE OF ACTION IN

#### OPEN WATER, MARINE AND FRESH WATER ENVIRONMENTS

It is important to differentiate the three types of bioremediation processes since their efficacy requires precise application parameters which vary in different types of environments. The limitations and decision points on usage have been covered extensively in previously issued materials but require more simplification, hence this guidance has been provided to simplify the decision making processes.

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Essential facts stated in the May 2000 NRT SCIENCE AND TECHNOLOGY COMMITTEE-Fact Sheet: Bioremediation in Oil Spill Response are:

"Several factors influence the success of bioremediation, the most important being the type of bacteria present at the site, the physical and chemical characteristics of the oil, and the oil surface area....

"Effective bioremediation requires that:

- (1) Nutrients remain in contact with the oiled material, and
- (2) Nutrient concentrations are sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation." i

#### NCP PRODUCT TYPES LISTED:

The Bioremediation Agent Types listed on the NCP Product Schedule are deliberately designated and appear as follows:



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“1. Microbiological Cultures (MC)

2. Nutrient Additives (NA)

3. Enzyme Additive (EA)”

The first type (MC) constitutes a bioremediation process that utilizes non-indigenous (foreign) bacteria. While useful in controlled environments, a prevailing concern with these types of products has been that the introduction of foreign species might cause future problems which may not

become apparent for some time. The second type, (NA) is those agents that contain nutrients or fertilizers to support the microorganisms present in the spill environment. Both are designated as not applicable for open water environments. See 2001 EPA Guidance Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands which extensively covers the usage of these two product types which need not be repeated here.

On the other hand, the third type is appropriate as a first response tool in open water environments. Bioremediation (EA) Type has evolved in recent years and has been the subject of considerable technological advances with wide applicability for oil spill response in fresh, brackish, marine and open water environments with temperature ranges as low as 28 degrees. The mode of action of this type will be covered in detail here.

## IMPORTANT CONTEXT

The reason for oil spill cleanup is to reduce or eliminate the toxic components, thus enabling the survival of fauna and flora including single cell organisms in each niche of the food chain. Although today's dispersants eliminate the visual and other damaging aspects of the spill on the surface, the spill's toxicity problem has remained in the environment and at times been worsened by the addition of further hydrocarbons in dispersants. The goal of the bioremediation process is to convert oil/hydrocarbon based material to CO<sub>2</sub> and water, thereby permanently removing oil/hydrocarbons from the environment and returning the affected spill area to the pre-spill conditions.

Herewith, the three main types of bioremediation are further defined along with their modes of action to help OSC's, federal, state, and local officials as well as responsible parties to understand and make more informed decisions about bioremediation agents when selecting appropriate oil spill response tools.

## CATEGORY TYPE ENZYME ADDITIVE (EA)

As covered, while NRT and RRT guidance addresses the (MC) and (NA) bioremediation types extensively in the 2001 Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands it does not sufficiently detail the mode of action of Bioremediation Type EA. iii Below are data to remedy this.

## ENZYMATIC AGENT DEFINITION:

Bio-catalysts designed to enhance the emulsification and/or solubilization of oil to make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

## (EA) TYPE MODE OF ACTION:

Enzyme Additive mode of action is applicable in open/moving water (fresh, salt and brackish), marsh/estuaries, shoreline and soil environments. When applied, the non-toxic converters and bio-surfactants in Bioremediation Agent (EA) Type eliminate the classic appearance of an oil spill by emulsifying and solubilizing the molecular hydrocarbon structure and eliminating the adhesion properties of crude oil. This usually takes place within the first 5 - 30 minutes (depending on temperature). The emulsified oil continues to float near the surface thereby eliminating a secondary impact to the water column and seabed.

With the toxicity and adhesion properties eliminated, wildlife that may come in contact with the broken down hydrocarbons they will not become coated in oil and oil adherence to marsh, shorelines, sands, and manmade structures is eliminated. The flammability is eliminated in a short time (depending on temperature) protecting ports, harbors and drilling rigs from the potential explosion hazards associated with fuel spills.

A further action of bioremediation category EA, (there are numerous enzymes contained in the product's matrices) is that the enzymes then attach themselves to the hydrocarbons with the biosurfactants, developing protein binding sites, that act as a catalyst to speed up the bioremediation process by inducing enhanced indigenous bacteria to utilize the detoxified oil/hydrocarbons as a food source. The EA category also contains properties that cause all the constituents to remain in contact with the spilled oil/hydrocarbons in moving waters.

Over the next few days or weeks (again, depending on temperature), non-toxic nutrients in the Enzyme Additive type rapidly colonize indigenous bacteria to large numbers. The colonized bacteria consume the detoxified hydrocarbon emulsion, digesting the spill to CO<sub>2</sub> and water, thereby

permanently removing the oil/hydrocarbons from the environment and resulting in final water clarification. Without category (EA) assistance, this natural process may take up to 20 years based on Ixtoc and the Valdez spill studies.

## SHORELINES/MARSHES:

When a spill has already made land fall or contaminated a marsh, category EA can be applied to lift the spill off the marsh grass (or sandy beaches and shorelines), limiting the time the spill can adversely impact these areas. The use of category EA does not deplete the O<sub>2</sub> from water since the spill is held on the surface utilizing predominantly atmospheric O<sub>2</sub>.

With category EA there are no tradeoffs or deleterious effects with this response method.

There is no limited window of opportunity for the application of category EA; it can be used in estuaries, in open (salt) water and, moving fresh water in rivers and soil. It is effective as a first response tool and/or when applied days or months after a spill. Category EA can even be applied to oil that is lying on the seabed floor as long as the product can be brought into contact with the oil which will eventually lift it to the surface returning the seabed to pre-spill conditions.

At the date of this writing, there is only one product on the NCP list that falls under this Bioremediation Agent Type EA classification: (B53-EA-OIL SPILL EATER II)

## CATEGORY TYPE MICROBIOLOGICAL

### CULTURE ADDITIVE (MC)

As covered in NRT Science and Technology Guidance;... bioaugmentation, is a process “in which oil degrading bacteria are added to supplement the existing microbial population.”.

#### MICROBIAL AGENTS DEFINITION:

Concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing medium that have been air- or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be grown-up in bioreactors at the spill site. All commercially available agents use naturally-occurring microorganisms. Some agents may also contain nutrients to assure the activity of their microbial cultures. This type of agent is intended to provide a massive inoculum of oil degrading microbes to the affected area thereby increasing the oil-degrading population to a level where the spilled oil will be used as a primary source of food for energy. Microbial agents are designed to enhance the biodegradation of oil at any, location and would be most useful in areas where the population of indigenous oil degraders is small.

#### (MC) TYPE MODE OF ACTION:

Bioremediation Agent Type (MC) mode of action utilizes non-indigenous bacteria with the objective to digest oil/hydrocarbons to CO<sub>2</sub> and water.

Bioaugmentation is considered a ‘polishing up’ or ‘finishing’ response product in that it cannot be applied to fresh oil because the toxicity levels kill the added oil degrading bacteria.

When non indigenous bacteria are placed on or near weathered oil these bacteria attempt to release enough quantities of biosurfactants to detoxify the spill so the oil-degrading bacteria will not be adversely impacted by the spill’s toxicity, enabling them to use the hydrocarbons as a food source.

The oil degrading bacteria (both indigenous and non indigenous) produce enzymes to develop protein binding sites which permits the bacteria to convert the molecular structure of the hydrocarbons for use as a food source. This process requires a protracted amount of time.

While bioaugmented bacteria acclimate to a spill site, the temperature of the water and or environment, the PH, and the available nutrients, these and other associated and variable environmental conditions may produce adversity that cannot be overcome. These factors along with the unknown time frames associated with their acclimation process are at least partially responsible for the past uncertainty associated bioremediation (MC) type as a viable cleanup methodology.

The application of non-indigenous bacteria generally must be performed where there is very little water movement. Water movement causes the products to dilute to ineffective levels that are unable to stave off the natural competition from indigenous bacteria, and, thus, will not be in sufficient

population numbers to produce enough biosurfactants and enzymes to start the breakdown of the molecular structure of the hydrocarbons for a food source. (Lab environments do not emulate this competitive environment; hence, particularly in any area of moving waters, the final outcomes are often uncertain.)

Next to the toxicity of the spill, the most difficult aspect of utilizing non-indigenous bacteria in a foreign environment is the natural competition from the indigenous bacteria that are already acclimated to the spill area; thus, they generally win out.

Bioaugmented bacteria developed specifically for fresh water must be used in fresh water settings only. Products containing salt water bacteria can only be utilized in salt water. (MC) Type is best used on closed and/or controlled environments and is not effective in open water environments.

The use of non indigenous bacteria in most countries is not permitted due to the uncertain effects of allowing non indigenous species in sensitive habits and environments.

## CATEGORY TYPE NUTRIENT ADDITIVE (NA)

As covered in NRT Science and Technology Guidance;... this next category (NA)--biostimulation is a process “in which nutrients, or other growth limiting substances, are added to stimulate the growth of indigenous oil degraders.”

#### NUTRIENT AGENTS DEFINITION:

Bioremediation Agents containing nitrogen and/or phosphorous as the primary means to enhance the rate of growth of indigenous oil-degrading microorganisms. This type of agent is intended to increase the oil-degrading biomass already present in an affected area to a level where the oil will be used as a primary source of food or energy. Because the natural environment may not have sufficient nutrients to encourage bacterial metabolism and growth, extra nutrients may be required. The purpose of this type of agent, therefore, is to provide the nutrients necessary to maintain or increase microbial activity and the natural biodegradation rate of spilled oil.

#### (NA) TYPE MODE OF ACTION:

The (NA) mode of action involves the general use of nutrients or fertilizers that contain various volumes of Nitrogen N and phosphorous P. The nutrients are placed in conjunction to a spill, where they are expected to enhance the growth and colonization of indigenous bacteria. These bacteria need time to secrete biosurfactants to attack the molecular structure of the spill by solubilizing the oil/hydrocarbons, then emulsifying the spill, increasing the oil-water interface to detoxify the hydrocarbons to the point the enhanced indigenous bacteria can utilize the spill as a food source.

It can be very difficult to apply nutrients or fertilizer in a spill area with toxic oil and still be able to enhance bacteria. Much of the indigenous bacteria are destroyed by the toxicity of the spill initially. Because of the toxicity of the oil, this situation usually precludes the nutrients or fertilizer being capable of enhancing what is left of the indigenous bacteria.

It is also challenging to supply nutrients or fertilizers in a concentration to enhance bacteria without increasing the nitrogen levels to the point that it becomes deadly toxic to aquatic life. An additional problem is getting the nutrients or fertilizers to stay with the oil especially on or in moving waters.

The process of enhancing indigenous bacteria with nutrients and fertilizer and waiting for them to secrete biosurfactants and enzymes in order to start the bioremediation process takes a protracted period of time making (NA) type inappropriate as a first response agent

Bioremediation category (NA) can be effectively used where there is little tidal flush, and where the oil has weathered so its toxicity is reduced to the point that indigenous bacteria can survive. This requires NA to be used only as a polishing up agent, with limited scope.

#### A BRIEF NOTE ON PHYTOREMEDIATION

Phytoremediation has been defined as the use of green plants and their associated microorganisms to degrade, contain, or render harmless environmental contaminants.

Phytoremediation of petroleum hydrocarbons generally involves three major mechanisms: (1) degradation, (2) containment and (3) the transfer of contaminants from soil to the atmosphere.

For further information on applicability consult page 87 of <http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf>

#### SUMMARY

The three types of bioremediation and their mode of actions as described above have been detailed here to help responders understand how these agents will interact with a spill. The different types and their mode of actions are clearly independent of each other, even though their end point in principle is the same; the ability to reach that end point, and the amount of time it takes to do so, is clearly different.

i Bioremediation [Types MC and NA] for open water spills is not considered to be appropriate or achievable because of the above two requirements. When nutrients are added to a floating slick, they immediately disperse into the water column, essentially diluting to background levels. [with the exception of NCP Listed Type EA based on extensive field use and testing on fresh and weathered hydrocarbons/oil. It recently demonstrated an 80% rate of PAH degradation on Macondo Block La. sweet crude containing Corexit per March 3 2011- BP BCST D.Tsao , LSU R.J. Portier, L. M. Basirico Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response.]

ii 2001 Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands (<http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf>)

iii This description of the EA Type mode of action is based on the NCP listed sole sourced product Oil Spill Eater II's field use and test documentation on fresh and weathered hydrocarbons/oil in ocean, fresh water and shoreline environments. If another EA Type product is added to the NCP List, these descriptions may not apply and should be validated in field tests with that product.

iv As per NRT Science and Technology Committee Bioremediation Fact Sheet: "Added bacteria seem to compete poorly with the indigenous population." ... "and has not been shown to have any long-term beneficial effects in shoreline cleanup"

#### References:

1. EPA: NRT SCIENCE AND TECHNOLOGY COMMITTEE Fact Sheet: Bioremediation in Oil Spill Response An information update on the use of bioremediation. May, 2001
2. Zhu X., Venosa A.D., Suidan M.T., (Sept 2001) Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands, U.S. EPA.
3. U.S. EPA (2012) NCP Product Schedule, <http://www.epa.gov/oilspill>  
<http://www.epa.gov/oem/content/ncp/products/oseater.htm>
4. BP BCST D.Tsao, LSU R.J. Portier, L. M. Basirico, March 3 2011, Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response.
5. Zhu X., Venosa A.D., Suidan M.T., (2004) EPA/600/R-04/075 Literature Review on the use of Commercial Bioremediation Agents for Cleanup of Oil-Contaminated Estuarine Environments.  
<http://www.epa.gov/oem/docs/oil/edu/litreviewbiormd.pdf>
6. Alleman, B.C., and E.A. Foote. 1997. Evaluation of Amendments for Enhancing Microbial Activity in Soils from Site 18 at MCAGCC Twentynine Palms,

California. Battelle, Columbus, OH. Performing Organization Report Number

D.O. 1795. Sponsoring Agency Report Number TCN 96-026. Feb. 7, 1997.

7. Zwick, T.C., Foote, E.A., Pollack, A.J., Boone J. L., Alleman, B.C., Hoeppel, R.E., Bowling L. (1997) Effects of nutrient addition during bioventing of fuel contaminated soils in an arid environment. In: In-Situ and On-Site Bioremediation: Volume 1, Battelle Press, Columbus, OH, pp. 403-409.

8. Bonner J.S., Autenrieth R.L., Microbial Petroleum Degradation Enhancement By Oil Spill Bioremediation Products, (Oct 1995) Report Submitted to Texas General Land Office (Comparative analysis of 13 NCP Listed Bioremediation Products, EA Type PAH reduction efficacy exceeded MC and NA.Types)

9. U.S. Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division 26 W. Martin Luther King Drive Cincinnati, OH--added bacteria seem to compete poorly with the indigenous population (Tagger et al., 1983; Lee and Levy, 1989; Venosa et al., 1992)--biostimulation alone had a greater effect on oil biodegradation than the microbial seeding (Jobson et al., 1974; Lee and Levy, 1987; Lee et al., 1997b, Venosa et al., 1996).

#### EA Type References:

10. LSU: Characteristics, Behavior, & Response Effectiveness of Spilled Dielectric Insulating Oil in the Marine Environment, June 2011

For U.S. Department of the Interior Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE) Herndon, VA; By Louisiana State University

Department of Environmental Sciences

[http://www.bsee.gov/uploadedFiles/BSEE/Research\\_and\\_Training/Technology\\_Assessment\\_and\\_Research/aa%283%29.pdf](http://www.bsee.gov/uploadedFiles/BSEE/Research_and_Training/Technology_Assessment_and_Research/aa%283%29.pdf)

11. A.T. Merski, (1993)-NETAC Oil Spill Response Bioremediation Agents, Evaluation Methods Validation Testing, Discussion of Results.

12. Dr. E. Brown, University of Alaska, Fairbanks (1990) Bioremediation performed on PAH's shows extreme or great reduction in the target analytes using EA Type. Report of Exxon tested Bioremediation EA Type in 1989 at Florham Park, New Jersey showing effective by a factor of better than 90% on the North Slope Alaskan Crude oil from the Valdez spill.

13. Galen Bartman, Oil Spill Eater Respirocity Evaluation CAI Lab. No. 3265 (July 1990) additive [EA] has a meaningful and significant effect on decreasing the oil concentration and increasing the oxygen take up.

14. US Marine Corps at 29 Palms utilizing EA remediated tank wash out and several types of fuels (including tetra ethal lead) to State of California acceptable levels, DOD Environmental Award Testing and Evaluation of Enzymatic Catalysis for the Remediation of Petroleum Contaminated Soils (Oct 93) pg 66 [http://osei.us/tech-library-pdfs/2011/OSEI%20Manual\\_FINAL-2011.pdf](http://osei.us/tech-library-pdfs/2011/OSEI%20Manual_FINAL-2011.pdf) 15. State of Alaska, Legal Closure Letter: pg75- 80 [http://osei.us/tech-library-pdfs/2011/OSEI%20Manual\\_FINAL-2011.pdf](http://osei.us/tech-library-pdfs/2011/OSEI%20Manual_FINAL-2011.pdf) The soils have been remediated to the most stringent cleanup levels-ADEC

16. State of New York, Groundwater remediation of heating oil by Alpha Geo Science with complete sampling and testing certified by NYSDEC, Summary and Results of In Situ Soil Remediation; No. 95-16786 pg. 80-86 [http://osei.us/tech-library-pdfs/2011/OSEI%20Manual\\_FINAL-2011.pdf](http://osei.us/tech-library-pdfs/2011/OSEI%20Manual_FINAL-2011.pdf)

17. R.H. Ward, SRI San Antonio Texas (1999)-EA Type does not sink oil into water column or sediments - Swirl Flask Dispersant Effectiveness Test SwRI Project Number: 08-2326-088 Workorder: 8783

18. Resource Analysts, Inc. Subsidiary of MILLIPORE (June 1990) References: 1) EPA SW 846, 3RD Edition Determination of no Trace Elements and Chlorinated hydrocarbons in EA Product.

19. M.en C. Gabriel Peneda Flores, Q.B.P. Norma Pescador Elizondo, (2002) Ecologia microbial Lab, University of Mexico-Instituto Politecnico Nacional, Escuela Nacional De Ciencias Biologicas -- Efficacy test of EA Type on heavy (Maya Crude) and medium weight crude oil demonstrates significant reduction of PAH's (54% reduction in 30 days on the Maya crude, and medium crude reduced 80% in 30 days.)

20. Environmental Protection Authority New Zealand, Hazardous Substances Division, Benjamin Sowman, (16 July 2012); SOS # 1001797; Determination of the Status of Oil Spill Eater II-Non Hazardous

21. Bioremediation Guidance Appendix \_\_\_\_ Bioremediation Agent EA Type Salt/Fresh Water Toxicity Tests

#### APPENDIX **Bioremediation Agent EA Type Salt Water Toxicity Tests** (EPA standard for toxicity of >100ppm = practically non-toxic)

\*richard, OSE II LC50 test 96hr mysid (LC50 of >1900) and 7 day mysid chronic

of 2500) tests, EPA/NETAC bioremediation protocol development, administrator for  
C Tom Merski (1992), EPA Research and Development Cincinnati Ohio,

, J E, and Jones J C, Evaluation of tier III bioremediation agent screening  
col for open water using commercial agents : preliminary report / toxicity tests  
OCLC number 206766502 Library EKCD Call number EPA/600-X-93,  
II Mysidopsis bahia static LC50 48hr 6,698 and 96 hr 5 970 static renewal LC50 48hr  
0, 96hr >5700, 7 day 2 500, Menidia beryline static LC50 48hr 8839, and 96hr  
ng/l

to Systems Division Resource Analysts Inc New Hampshire Batch 329, OSE II toxicity  
Mysidopsis Bahia LC50 96hr 2100mg/l, March 9 1990

thy Ward, Robert Boeri, Enviro Systems Division, Resource analysts New  
shire, batch 9820 for OSE II on the EPA dispersant toxicity test Artemia Salina, LC50  
r >100, 48hr LC50 >100mg/l, fuel oil 48hr LC50 12 6 mg/l, OSE II and Fuel oil 48hr  
) 29 4mg/l, October 1990

### **EA Type Fresh Water Toxicity Test**

is M, Environment Canada Spilltox Environmental Technology Centre  
<http://www.etccte.ec.gc.ca/databases/SpillTox/Default.aspx> (key word OSE II)  
onment Canada OSEII Daphnia magna 48 LC50 >10000  
, Oncorhynchus mykiss 96 LC50 >10000 mg/L, Photobacterium  
phoreum 5 IC50 = 5109 mg/L, Photobacterium phosphoreum 25 IC50  
'4 mg/L, Photobacterium phosphoreum 083 IC50 = 7952 mg/L,  
17, 1993) Biological test method: acute lethality test using rainbow trout Environment  
da, Conservation and Protection, Ottawa, Ontario Report EPS 1/RM/9, 51 pp

1 D Bio-Aquatic Testing Inc Carrolton Tx (214) 241-5928, Client BO-12-91-2239  
II, toxicity test 48hr LC50 Pimephales Promelas 9300mg/l, (l, December 1991)

er B, Huther and Associates Denton, Texas, (940) 387-1025, for Kwang Keun Kim  
1 Korea project 05457, OSE II, Toxicity test Pimephales Promelos (minnows)  
1 5856 34mg/l, (l June 2008)

er B, Huther and Associates Denton, Texas (940) 387-1025, for Kwang Keun Kim  
1 Korea project 05457, OSE II LC50 Toxicity test Ceriodaphnia Dubia (water flea) 24hr LC50  
000mg/l, (l June 2008)

Sincerely,  
Steven Pedigo

P.S. We still have open questions from previous correspondence and I will have detailed answers to your questions  
soonest. If you could please provide answers to the 13 questions in my last email to you which some of my answers  
depend upon.